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Ecology and Population Biology of Aflatoxigenic Fungi in Soil

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ABSTRACT

Soil serves as a reservoir for *Aspergillus flavus* and *A. parasiticus*, fungi that produce carcinogenic aflatoxins in agricultural commodities. Populations in soil are genetically diverse and individual genotypes show a clustered distribution pattern within fields. Surveys over large geographic regions suggest that climate and crop composition influence species density and aflatoxin-producing potential. Aflatoxigenic fungi reside in soil as conidia, sclerotia and hyphae, which act as primary inocula for directly infecting peanuts or for infecting aerial crops (corn,

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cottonseed, tree nuts) through wind and insect dispersal. Infected crops periodically replenish soil populations during drought years.

Key Words: Aflatoxin; *Aspergillus flavus*; *Aspergillus parasiticus*; Conidia; Corn; Cotton; Genetic diversity; Mycotoxins; Peanut; Sclerotia; Soil.

INTRODUCTION

Species belonging to *Aspergillus* section *Flavi* are among the most intensively studied of all fungi, due largely to their formation of carcinogenic aflatoxins in agricultural commodities that impact animal and human health (Hussein and Brasel, 2001; Peraica et al., 1999). Apart from the health concerns of aflatoxins, the economic cost of these mycotoxins is enormous, amounting to approximately $25 million annually to the peanut industry in the southeastern United States alone (Lamb and Sternitzke, 2001). Aflatoxigenic fungi are common components of soil mycobiota and are actively involved in decomposition and nutrient cycling (Klich et al., 1992; White and Johnson, 1982). Members of section *Flavi* utilize a wide range of carbon and nitrogen sources (Davis et al., 1967; Hesseltine et al., 1970a) and produce a diversity of enzymes for degrading plant components such as cellulose, pectin, lignin and lipids (Betts and Dart, 1989; Cotty et al., 1990; Long et al., 1998; Olutiola, 1976). These fungi also invade developing seeds of crops, and the primary inoculum for infection originates from soil. Therefore, an understanding of the activities and population structure of aflatoxigenic fungi in soil is a prerequisite for developing effective measures to control aflatoxin contamination.

AFLATOXIGENIC SPECIES

Relatively few fungi are capable of synthesizing aflatoxins but two species, *A. flavus* and *A. parasiticus*, are widespread and important colonists of agricultural commodities. *A. flavus* commonly contaminates corn, peanuts, cottonseed and tree nuts with aflatoxins before harvest and during storage (Diener et al., 1982, 1987; Payne, 1998; Schroeder and Boller, 1973; Siriacha et al., 1989). The species typically produces aflatoxins B_1 and B_2 and cyclopiazonic acid (Horn et al., 1996) and is extremely variable in mycotoxin production, with strains ranging in toxigenicity from nonproducers to potent producers of aflatoxins (Horn and Dorner, 1999). In contrast, *A. parasiticus* is most prevalent in peanuts and synthesizes

aflatoxins G_1 and G_2 in addition to the B aflatoxins but not cyclopiazonic acid (Dorner et al., 1984; Horn et al., 1996). *A. parasiticus* generally produces high levels of aflatoxins and nonaflatoxigenic strains are rare (Horn et al., 1996; Tran-Dinh et al., 1999).

A. flavus as a species is genetically complex and has been subdivided into several groups based on morphology, mycotoxin profile and molecular characters. Morphologically, *A. flavus* isolates can be categorized either as the typical L strain with sclerotia >400 μm in diameter or as the S strain which is dominated by abundant small sclerotia <400 μm in diameter (Cotty, 1989). For the purposes of this review, mention of *A. flavus* will imply the L strain unless otherwise specified. The S strain was first recognized as an undescribed taxon by Hesseltine et al. (1970b) and was formally described as variety *parvisclerotigenus* by Saito and Tsuruta (1993). The type culture of *A. flavus* var. *parvisclerotigenus* produces only B aflatoxins, but other S-strain isolates produce both B and G aflatoxins (Cotty and Cardwell, 1999; Geiser et al., 2000; Hesseltine et al., 1970b; Novas and Cabral, 2002; Saito and Tsuruta, 1993). Only S-strain isolates that produce B aflatoxins have been identified from the United States; both types of the S strain occur in Argentina, West Africa, Southeast Asia and Australia.

Based on RAPD analyses and sequencing of gene regions, *A. flavus* is nonmonophyletic and is separable into two genetic groups that cannot be readily distinguished morphologically (Geiser et al., 1998, 2000). Group I consists of both L and S strains that produce only B aflatoxins and group II comprises S strains that produce B or B + G aflatoxins. Research on the population biology of aflatoxigenic fungi is dependent upon the accuracy of species identifications and the recognition of important intraspecific genetic groups. The current taxonomic uncertainty may to some degree compromise population studies and is reminiscent of earlier studies in which *A. flavus*, *A. parasiticus* and perhaps other species in section *Flavi* were lumped together as ''*A. flavus* group'' and as a consequence, much valuable information was lost.

Sterigmatocystin, an intermediate in the aflatoxin pathway, is synthesized as an end-point metabolite by several *Aspergillus* species (including *A. nidulans*) and even by representatives of other fungal genera (Barnes et al., 1994; Cole and Cox, 1981). Since this initial portion of the aflatoxin pathway is fairly widespread, it is not surprising that other aflatoxigenic species have been discovered in recent years. *A. nomius* is very similar to *A. flavus* morphologically but produces both B and G aflatoxins and is quite distinct in molecular characters from other members of section *Flavi* (Kurtzman et al., 1987; Peterson et al., 2001). This species parasitizes insects and also has been isolated from soil. Another species,

A. bombycis, has been isolated (along with *A. nomius*) from insect frass in silkworm-rearing houses in Japan and Indonesia (Peterson et al. 2001). *A. pseudotamarii* was described from two isolates from Japan and Brazil (Goto et al., 1997; Ito et al., 2001) and *A. ochraceoroseus*, the only species outside of section *Flavi* reported to produce aflatoxins, has been isolated only from African forest soils (Klich et al., 2000; Maggi and Persiani, 1983). Unlike *A. flavus* and *A. parasiticus*, these aflatoxigenic species, although interesting, do not have a major impact on agriculture.

GENETIC DIVERSITY OF SOIL POPULATIONS

The high diversity within *A. flavus* populations as revealed by colony morphology in the laboratory has long been recognized. Isolates differ in phenotype according to sclerotium production (nonsclerotial to predominantly sclerotial), conidial head formation (densely sporulating to mostly mycelial) and conidial color (bright yellow green to dark green) (Horn et al., 1996; Klich and Pitt, 1988; Raper and Fennell, 1965). The wide range in the production of aflatoxins and cyclopiazonic acid by *A. flavus* isolates is equally reflective of this variability (Horn and Dorner, 1999; Joffe, 1969; Schroeder and Boller, 1973).

Vegetative compatibility reactions within a population are another measure of diversity in aflatoxigenic fungi. In *Aspergillus* species, hyphal anastomosis between two individuals is genetically controlled by a series of *het* loci in which the occurrence of different alleles at one or more loci results in incompatibility (Leslie, 1993). Vegetatively compatible individuals within a population together form subpopulations called vegetative compatibility groups (VCGs). Papa (1986) first examined the diversity of *A. flavus* VCGs in corn kernels from 15 counties in Georgia, USA. Subsequent studies involved VCG analyses of soil populations of *A. flavus* in a cotton field from Arizona (Bayman and Cotty, 1991) and in a peanut field from Georgia (Horn and Greene, 1995) (Figure 1). In *A. flavus* isolates from the latter study, DNA fingerprints were unique for each VCG and were generally identical for isolates within a VCG, providing an independent confirmation of the use of VCGs as a measure of genetic variability within populations (McAlpin et al., 2002). Genetic diversity in *A. flavus* soil populations is very high, even within a small soil sample, and VCG or genotype diversity values (number of VCGs or genotypes divided by total number of isolates) range from 0.49 to 0.84 (Bayman and Cotty, 1991; Horn and Greene, 1995; Papa, 1986; Wicklow et al., 1998). DNA-fingerprint, RAPD and VCG analyses of *A. parasiticus* soil populations from Australia and the United States indicate that this species also is highly

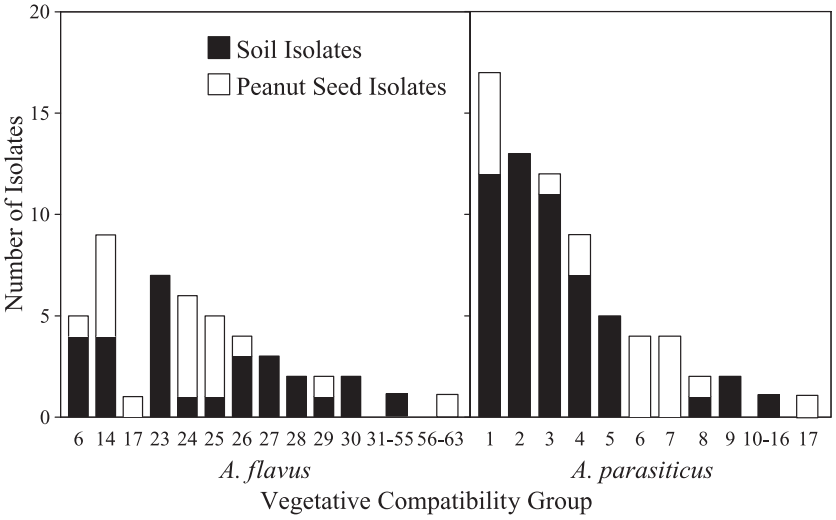

Figure 1. Diversity of *A. flavus* and *A. parasiticus* VCGs from a single peanut field in Georgia, USA. Horn and Greene (1995).

diverse genetically (Carter et al., 1998; Horn and Greene, 1995; McAlpin et al., 1998) (Figure 1).

Most of the phenotypic diversity in populations of *A. flavus* and *A. parasiticus* can be attributed to differences among VCGs. Morphological characters (sclerotium number, size and shape; conidial color) and mycotoxin production (aflatoxin B_1; cyclopiazonic acid) are more similar within a VCG than between VCGs (Bayman and Cotty, 1993; Horn et al., 1996; Novas and Cabral, 2002) (Figure 2). Aflatoxigenic and nonaflatoxigenic strains of both species are segregated into separate VCGs and are rarely found together in the same VCG (Bayman and Cotty, 1993; Horn et al., 1996).

VCGs of *A. flavus* and *A. parasiticus* appear to be widely distributed. *A. parasiticus* VCG 1 and several nontoxigenic *A. flavus* VCGs have been isolated from agricultural soils from across a large section of the United States (Horn and Dorner 1998, 1999). These distributions may reflect long-range dispersal or may be due to introductions from planting infected seed. It is not known to what degree VCGs comprise individuals that are clonal and genetically identical. In several instances, vegetatively compatible isolates of *A. flavus* have been shown to differ genetically (Bayman and Cotty, 1993; McAlpin et al., 2002).

The origins of genetic diversity in soil populations of aflatoxigenic fungi and the selective forces that maintain this diversity are not under-

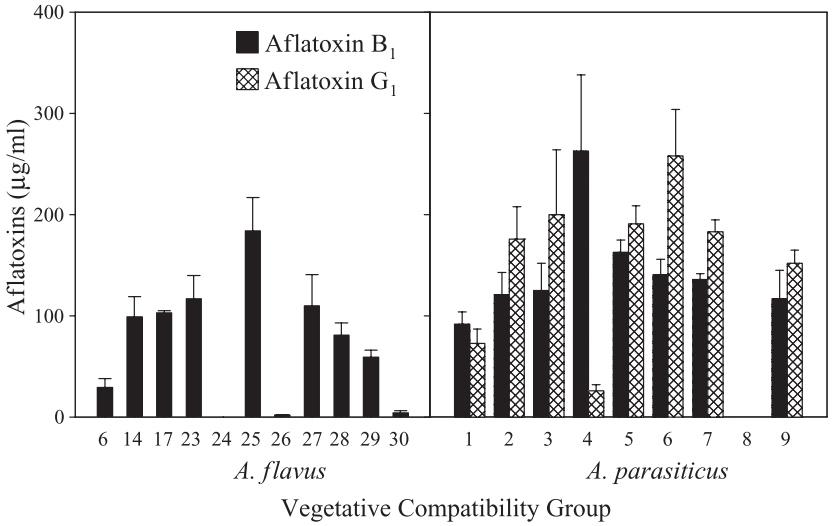

Figure 2. Production of aflatoxins B_1 and G_1 within VCGs of *A. flavus* and *A. parasiticus*. Standard deviations are indicated. *A. parasiticus* VCG 8 produces 226 ± 23.6 μg/mL *O*-methylsterigmatocystin (intermediate in the aflatoxin pathway). Horn et al. (1996).

stood. *A. flavus* and *A. parasiticus* have no known sexual stage. The high VCG diversity in soil suggests that heterokaryosis, which occurs only between vegetatively compatible individuals, is not common. The parasexual cycle has been reported for *A. flavus* and *A. parasiticus* (Papa 1973, 1978) and could result in mitotic recombination, but this cycle requires heterokaryosis as a precondition and has not been demonstrated in *Aspergillus* species outside of the laboratory. Geiser et al., (1998) have proposed that *A. flavus* has a population structure indicative of recombination based on a lack of congruence of five gene trees. Similar evidence for recombination has been reported for *A. nomius* (Peterson et al., 2001). Data suggesting cryptic recombination do not explain the method of recombination, how often recombination occurs, or when recombination occurred in the history of species.

GEOGRAPHIC DISTRIBUTION

Patterns in the distribution of *A. flavus* and *A. parasiticus* in soil have been observed at spatial scales ranging from single fields to large

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geographic regions and have even been examined, to a limited extent, on a worldwide basis. These distribution patterns reflect differences in propagule density, species incidence, and degree of toxigenicity.

Regional Surveys

Extensive sampling of single fields have shown that *A. flavus* and *A. parasiticus* are not randomly distributed in soil but rather, show an aggregated form of distribution based on propagule density (Griffin et al., 1981, 2001) and VCG incidence (Horn and Greene, 1995). Localized colonization of soil substrates by aflatoxigenic fungi may create patches of higher propagule density, particularly if accompanied by extensive sporulation. Similarly, colonization by a single fungal individual would result in clonal aggregations of an identical VCG.

Orum et al. (1997, 1999) examined soil populations of *A. flavus* S strain in cotton fields within a relatively small geographic area in Yuma County, Arizona, USA. Variances in S-strain incidence and density at different spatial scales of sampling (20–100 km; 10–15 km; 1–5 km; 150–300 m) were compared and found to be highest among fields (1–5 km scale) (Orum et al., 1997). Subsequent sampling of fields adjacent to the original fields and with different crop histories revealed similar S-strain incidences, suggesting that patches extended beyond field boundaries and that factors other than crop composition and agricultural practices were responsible for the distribution patterns (Orum et al., 1999). Analyses of soil populations in Arizona were further complicated by the rapid changes in S-strain incidence from one year to the next, particularly within a 30-km^2 area of the study in which populations may have been influenced by weather-related factors. Bayman and Cotty (1991) similarly showed that the profile of *A. flavus* VCGs in soil from a cotton field shifted dramatically during consecutive years, and the dominant VCG during one year was not detected in the following year. Current knowledge of the population dynamics of aflatoxigenic fungi cannot definitively explain these fluxes in genotype composition. Rapid population changes may be due to large-scale dispersal of a genotype into an area or to selection of a particular genotype under specific crop conditions, or the apparent changes may simply reflect nonrepresentative sampling of the population.

Aflatoxigenic fungi show vertical patterns of distribution in agricultural soils, the details of which are largely uncharacterized. Conidia of *A. flavus* and *A. parasiticus* are extremely hydrophobic and when applied to the soil surface, do not readily move downward beyond the upper 6 cm of soil despite repeated rainfall events (Horn et al., 2001). Nevertheless, sizable populations of species from section *Flavi* are present in cultivated fields up

to 30 cm in depth (Horn et al., 2001; Pettit et al., 1973), possibly due to the mixing of soil during plowing or to colonization of organic matter at such depths.

Continental Surveys

Few single research projects have attempted large-scale comparisons of soil populations of aflatoxigenic fungi over geographic areas that encompass differences in climate, soil type and cropping systems. The most studied area, the southern United States, is characterized by high temperatures and periodic droughts during the growing season and, therefore, has a high incidence of aflatoxin contamination in susceptible crops.

Horn and Dorner (1998) established a 3300-km transect from eastern New Mexico to northern Virginia in the southern United States and examined soil populations in peanut fields from four major peanut-growing regions as well as fields with other crops in regions where peanuts are not cultivated (Figure 3). *A. flavus* was the dominant aflatoxigenic fungus across the transect; the L strain was present in all regions whereas the S strain was more restricted in distribution and was most prevalent in the cotton-growing regions of Louisiana and the eastern half of Texas. Fields from peanut-growing regions had significantly higher densities and incidences of *A. parasiticus* than fields from regions where other crops are traditionally grown, not an unexpected finding since *A. parasiticus* infects peanuts more than aerial crops such as corn and cotton (Diener et al., 1987; Hill et al., 1985). *A. nomius* was detected at low incidences only in the Mississippi Delta region of Louisiana and Mississippi. Positive correlations in soil density between species from section *Flavi* as well as between those species and total filamentous fungi suggest that environmental factors were affecting fungal populations as a whole and that regional differences could not be easily explained by climate alone. In another study, soil populations were characterized from three cotton-growing regions of southeastern (Louisiana, Mississippi, Alabama) and southwestern (Arizona) United States (Cotty, 1997). The incidence of *A. flavus* S strain was high in the Mississippi Delta region, as reported in the previously described transect, and in Arizona.

Supplementing these surveys of the southern United States with other more limited studies from the literature to cover additional regions of the country may broaden our understanding of distribution patterns, although doing so introduces inaccuracies arising from differences in sampling techniques, medium composition, incubation conditions and taxonomic interpretations. Nevertheless, trends in fungal distribution can be discerned, particularly for *A. parasiticus*. Soil populations of *A. parasiticus* are asso-

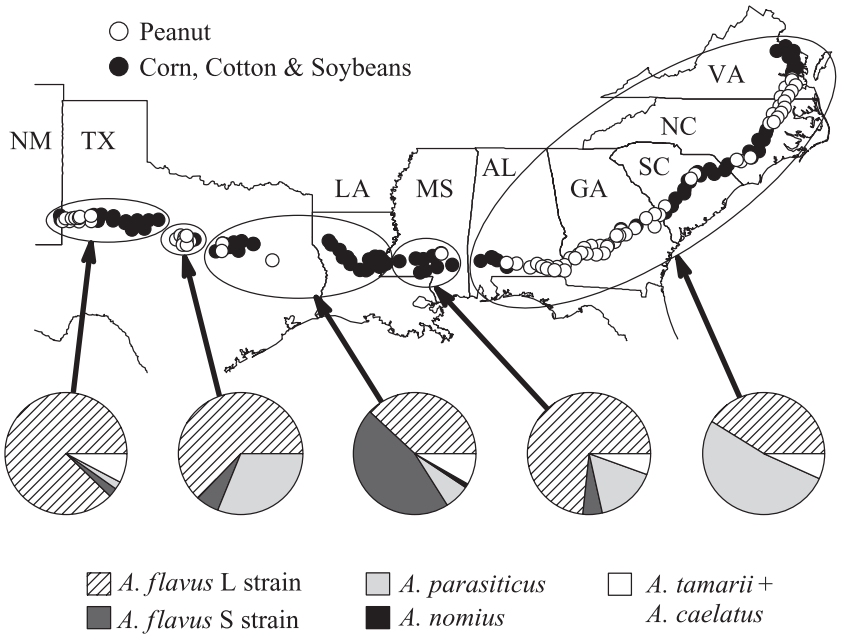

Figure 3. Incidences of species (as percentages of *Aspergillus* section *Flavi*) in soil along a transect in the United States. *A. tamarii* and *A. caelatus* are nonaflatoxigenic species. State abbreviations: NM, New Mexico; TX, Texas; LA, Louisiana; MS, Mississippi; AL, Alabama; GA, Georgia; SC, South Carolina; NC, North Carolina; VA, Virginia. Horn and Dorner (1998).

ciated with peanut cultivation in the southeastern United States (Horn and Dorner, 1998), but such an association is not apparent in other areas of the country. Horn and Dorner (1998) reported sizable *A. parasiticus* populations in Virginia north of the peanut-growing region, and the species also has been reported from soils in pistachio and fig orchards from California (Doster and Michailides, 1994; Doster et al., 1996) and in corn fields from the Midwest (Angle et al., 1982; McAlpin et al., 1998; Shearer et al., 1992; Wicklow et al., 1998). *A. parasiticus* has not been detected in cotton fields from the desert regions of southern Arizona (Boyd and Cotty, 2001; Cotty, 1997), and the species does not survive beyond one year when added to soil in that region (Wilson et al., 1996). Therefore, crop composition appears to greatly influence *A. parasiticus* populations in the southern United States but climate may be a more important determinant in other regions.

Several studies outside of the United States have shown geographic patterns in the distribution of aflatoxigenic fungi. A survey of soil populations from northern Japan southward into Indonesia indicated that *A. flavus* and *A. parasiticus* are more prevalent in the southern regions (Manabe et al., 1976; Manabe and Tsuruta, 1978). In the Republic of Bénin, West Africa, average yearly rainfall decreases with increasing latitude (Cardwell and Cotty, 2002). In association with this moisture gradient, *A. flavus* L-strain densities in soil from cornfields were significantly higher in the southern, wetter regions whereas densities of the S strain were higher in the northern, drier regions next to the Sahara Desert.

The aflatoxin-producing potential of *A. flavus* populations appears to be correlated with latitude. Soil populations of *A. flavus* are predominantly aflatoxigenic in the southern United States, with >95% of isolates producing aflatoxins in the peanut-growing region of southern Alabama and Georgia (Horn and Dorner, 1999) (Figure 4). In contrast, aflatoxin-producing

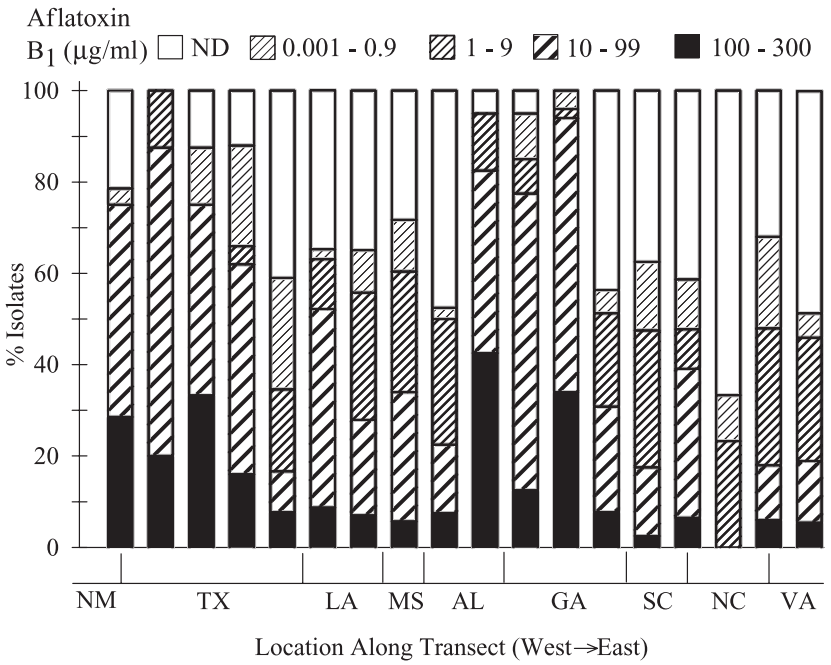

Figure 4. Production of aflatoxin B_1 by L-strain isolates of *A. flavus* from agricultural soils along a transect extending from New Mexico to Virginia, USA. See Figure 3 for map of transect and abbreviations of states. ND, not detected. Horn and Dorner (1999).

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isolates of *A. flavus* often comprise <50% of soil isolates at the higher latitudes of the Midwestern corn belt (McGee et al., 1996; Shearer et al., 1992; Wicklow et al., 1998). The least aflatoxigenic populations among four major peanut-growing regions in the United States were in the northernmost area of North Carolina and Virginia (Horn and Dorner, 1999). Cotty (1997) also reported that *A. flavus* toxigenicity is negatively correlated with latitude. A similar pattern in distribution has been shown for Japan where increasing latitude is associated with lower aflatoxigenicity of *A. flavus* populations (Manabe et al., 1976). Decreasing temperature with increasing latitude may be the common environmental variable associated with these observations.

Worldwide Surveys

Many *Aspergillus* species from section *Flavi* are distributed worldwide and have been found wherever soil fungi have been examined. *Aspergillus* species in general appear to be most abundant in subtropical and warm

Table 1. Soil populations of *A. flavus* and *A. parasiticus* from cultivated fields and neighboring forests in southwestern Georgia, USA.

	A. flavus		*A. parasiticus*	
Habitat	Density (CFU/g)[a]	Relative density (%)[b]	Density (CFU/g)	Relative density (%)
Cultivated fields				
Peanut	1286	0.88	909	0.62
Peanut	432	0.51	358	0.42
Peanut	194	0.20	427	0.43
Corn	782	0.25	1112	0.36
Corn	152	0.20	90	0.12
Corn	84	0.08	161	0.15
Neighboring forests				
Oak	ND[c]	–	32	0.007
Oak/sweetgum	32	0.006	ND	–
Oak/hickory	16	0.004	16	0.004
Pine	16	0.015	16	0.015
Pine	ND	–	ND	–
Pine	ND	–	ND	–

[a]Colony-forming units per gram of soil (dry wt.).
[b]Relative density = percentage of total filamentous fungi.
[c]ND = not detected.

temperate regions, particularly in agricultural soils, and decrease in density and species diversity with increasing latitude (Christensen and Tuthill, 1985; Klich et al., 1992). Klich (2002a) compiled data from over 100 studies and examined the global distribution of species within section *Flavi*. *A. flavus* occurred at greater than expected frequencies at 26–35° latitude (subtropical to warm temperate) and *A. parasiticus* occurred at slightly greater than expected frequencies in the tropics (0–15°). Because *A. flavus* was present at expected frequencies in all biomes (forest, grassland, wetland, desert and cultivated), it was suggested that temperatures optimal for growth (25–40°C) instead of habitat might account for the high frequency of this species at subtropical to warm-temperate latitudes (Klich, 2002a). However, data were based wholly on the occurrence of species (rare and common species were given equal weights) in published studies. A compilation that incorporates the absolute and relative densities of species may greatly modify these conclusions. Certainly *A. flavus* densities are much higher in cultivated fields than in natural habitats such as forests (Table 1) and prairies (Angle et al., 1982), and desert ecosystems are known to harbor high populations of *A. flavus* (Boyd and Cotty, 2001).

ADAPTATIONS FOR SURVIVAL IN SOIL

A. flavus and *A. parasiticus* are well adapted for survival in soil and may exist as conidia, sclerotia or hyphae. Identification of hyphae and conidia in soil, either by direct observation or indirectly through dilution plating, presents many difficulties (Klich et al., 1992). Aspergilli sporulate profusely in nature and colonies arising from dilution plating, a widely used technique for assessing soil populations, likely arise from quiescent conidia. Therefore, population statistics obtained through dilution plating suggest the potential of these fungi for colonization of plants and other substrates but say little about their current activity in soil.

Conidia of aflatoxigenic fungi when added to soil slowly lose their viability (Griffin, 1969; Horn et al., 1994). Wicklow et al. (1993) compared conidium viability of *A. flavus* and *A. parasiticus* for 3 years between northern (Illinois) and southern (Georgia) regions of the United States. *A. flavus* conidia were not detected in soil from either location by the end of the experiment; surprisingly, *A. parasiticus* conidia remained largely viable in Illinois but not in Georgia. The high soil densities of aflatoxigenic fungi in Georgia despite the increased conidium mortality may be due to the addition of large quantities of inoculum from crops contaminated with aflatoxins, the result of high temperatures and frequent droughts in the southern United States (Wicklow et al., 1993). Thus, population fluxes in soil reflect

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conidium mortality that is countered by the influx of inocula from infected crops and/or colonization of organic matter (Horn et al., 1994).

Sclerotia are commonly produced by strains of *A. flavus* and *A. parasiticus* in culture (Cotty, 1989; Horn et al., 1996; McAlpin et al., 1998; Shearer et al., 1992; Wicklow et al., 1998) and likely serve as resistant structures for surviving adverse environmental conditions (Coley-Smith and Cooke, 1971). Although the sclerotia are morphologically similar to stromata of *Petromyces alliaceus* (anamorph = *A. alliaceus*), a species in section *Flavi* that sporadically produces ascospores within stromata (Klich, 2002b), a sexual stage has never been linked to *A. flavus* and *A. parasiticus* sclerotia. Wicklow et al., (1993) demonstrated that the majority of sclerotia survived burial in Illinois and Georgia for 3 years; survivability was less on the soil surface (Wicklow, 1987). Sclerotia of *A. flavus* have been reported from nature on preharvest corn kernels (Wicklow et al., 1984) and in the pith of corncobs that had over wintered on the soil surface (Zummo and Scott, 1990). In addition, *A. parasiticus* sclerotia have been detected on insect-damaged peanut seeds (Horn et al., 1994), and large numbers of the small sclerotia of *A. flavus* S strain can form in developing cotton bolls (Garber and Cotty, 1997).

The mode of infection by sclerotia of aflatoxigenic fungi and their importance in agricultural ecosystems are poorly understood. Sporogenic germination in which conidial heads form on the sclerotium surface has been demonstrated under laboratory conditions and on the soil surface of a cornfield (Wicklow and Donahue, 1984; Wicklow and Wilson, 1986). Soil densities of *A. flavus* and *A. parasiticus* greatly increase in the vicinity of buried sclerotia, presumably due to conidia produced by sporogenic germination (Wicklow et al., 1993). Sclerotia of both species also may germinate in soil by producing mycelium and thereby directly invade the substrate. Stack and Pettit (1984a) reported colonization of organic matter through myceliogenic germination of *A. flavus* sclerotia. Similarly, peanuts became highly infected with *A. parasiticus* when soil in experimental control plots was inoculated with sclerotia (Horn et al., 1994). Without any evidence of sporogenic germination, it was surmised that infection likely occurred through invasion by mycelium from germinating sclerotia.

Wicklow et al., (1984) documented the dispersal of sclerotia onto soil during combine harvesting of five cornfields in the southern United States. Small numbers of *A. flavus* sclerotia were recovered from insect-damaged kernels before harvest and from chaff and debris exiting the combine exhaust. Extensive sampling of soil following harvest resulted in the recovery of only two sclerotia from the most heavily infected field. Since soil from those same fields contained high densities of *A. flavus* propagules (700–7400 CFU/g) that presumably represent conidia and hyphae, the

importance of sclerotia may be minimal. Sclerotia may instead be of greater importance in natural habitats or fallow fields where soil populations of aflatoxigenic fungi are very low and where preferred substrates such as seeds are either rare or not immediately available.

SOIL AS A SOURCE OF PRIMARY INOCULUM

Soil serves as a reservoir for primary inoculum that is responsible for the infection of crops susceptible to aflatoxin contamination. The aerial fruiting of crops such as corn, cotton and tree nuts dictates important differences in the manner of infection compared to the subterranean fruiting of peanuts (Payne, 1998).

Aerial crops become infected by *A. flavus* conidia that are dispersed by wind and vectored by insects, though it is often difficult to determine whether the conidia originated as primary inoculum from soil or as secondary inoculum from currently infected crops. Sporulation on crop debris deposited on the soil surface is clearly one source of inoculum. This has been demonstrated experimentally through biological control in which nontoxigenic strains of *A. flavus* and *A. parasiticus* sporulate profusely on inoculated grain that has been distributed onto the soil surface. Corn and cottonseed become infected with nontoxigenic strains, which reduce aflatoxin contamination by competing with native aflatoxigenic strains (Cotty, 1994; Dorner et al., 1999). Olanya et al. (1997) showed that *A. flavus* sporulates on waste corn deposited on the soil surface, creating a linear dispersal gradient of airborne conidia away from the corn deposits. Secondly, windborne dust containing *A. flavus* conidia may directly infect crops. Cotton bolls in Arizona became contaminated with aflatoxins when soil was artificially blown over the crop (Lee et al., 1986), and dust associated with disking has been implicated in infection of pistachio nuts (Doster and Michailides, 1994). Finally, insects disperse conidia of aflatoxigenic fungi directly from soil to the crop. Soil insects in cornfields harbor *A. flavus* and *A. parasiticus* both externally and internally (Lillehoj et al., 1980). Lussenhop and Wicklow (1990) showed that nitidulid beetles are involved in infection of corn ears in the southern United States. Buried ears that had overwintered supported visible *A. flavus* sporulation and were associated with nitidulid beetles, 68% of which were contaminated with *A. flavus* at the time of corn silking. The beetles were attracted to developing ears wounded by other insects such as the corn earworm, and ears became infected with a phenotypically distinct strain of *A. flavus* transmitted from the buried ears.

In contrast to aerial crops, peanuts are infected through direct contact with soil. Seeds from visibly undamaged pods become infected through a

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little-understood route of invasion involving hyphal penetration of the pod pericarp (Cole et al., 1986, Frank et al., 1994; Xu et al., 2000). In the more frequent mode of infection, pods and seeds are damaged by various arthropods and become highly susceptible to invasion by aflatoxigenic fungi. Larvae of the lesser cornstalk borer (*Elasmopalpus ligosellus*) are responsible for the scarification and penetration of pods in the southeastern United States (Bowen and Mack, 1993; Lynch and Wilson, 1991) whereas in tropical regions of the world, white grubs (scarab beetle larvae), termites and millipedes may cause considerable damage (Lynch et al., 1986, 1997; Umeh et al., 2000). These arthropods are most damaging to peanut pods in dry soil at elevated temperatures, conditions that are also conducive to drought stress in peanut plants. Drought-stressed peanuts have a lowered resistance to invasion by aflatoxigenic fungi due to cessation of phytoalexin production (Dorner et al., 1989).

The strong correlation between insect damage and aflatoxin contamination in peanuts is well established, but the sources of inoculum and their relative importance require further study. Direct exposure of the freshly created wound site to surrounding soil would allow for invasion by *A. flavus* and *A. parasiticus*. Conidia of these fungi are normally quiescent in soil, even within the geocarposphere of developing peanut pods (Griffin, 1969, 1972). Injury to the pod stimulates the germination of *A. flavus* conidia in soil adjacent to the wound (Griffin, 1972) due to release of sugars and amino-N compounds (Hale and Griffin, 1976). Wound sites on peanut seeds also may become infected from inoculum transmitted by arthropods. Lesser cornstalk borer larvae and fungus-feeding mites harbor *A. flavus* conidia both externally and internally and vector them to peanuts (Aucamp, 1969; Bowen and Mack, 1993). Dispersal by these arthropods in peanuts is likely for only limited distances in the soil environment compared to the potentially long-range distances by flying insects in aerial crops. Lesser cornstalk borer larvae migrate for short distances and have been captured in pitfall traps between peanut rows (Johnson and Smith, 1981; Jones and Bass, 1979). However, the internal incidences of surface-sterilized larvae are often low (<10%) (Bowen and Mack, 1993), suggesting that *A. flavus* is incidental from the soil environment and does not comprise secondary inoculum from neighboring infected pods. Detailed monitoring of the movement of individual larvae in soil during feeding is needed to clarify the association between larval damage and colonization by aflatoxigenic fungi.

Demonstration of the effect of soil population density on crop infection by aflatoxigenic fungi under field conditions has proven difficult, particularly with aerial crops where primary inoculum cannot be easily identified. Peanuts appear to be relatively insensitive to propagule density.

Griffin and Garren (1974) estimated that pod infection is possible with as few as 2.0 propagules of *A. flavus* in a 0.5-mm layer of the geocarposphere. Furthermore, an increase in soil density of *A. parasiticus* conidia from 100 to 10^5 CFU/g resulted in an increase of only 5% in the incidence of seed infection in drought-stressed peanuts (Horn et al., 1994). Drought and elevated soil temperatures responsible for increased peanut susceptibility to invasion by *A. flavus* and *A. parasiticus* may be more important than soil propagule density in determining the severity of peanut seed colonization.

SOIL POPULATION DYNAMICS IN RELATION TO CROPS

Soils from mesic habitats such as forests and prairies harbor very low populations of aflatoxigenic fungi compared to cultivated fields from the same areas, indicating that agricultural practices greatly increase soil populations (Angle et al., 1982) (Table 1). Pettit et al. (1973) reported that when peanuts were grown continuously on previously nonagricultural land, *A. flavus* was detected in soil by the latter part of the second year. Agricultural soils planted in annual crops are characterized by considerable exposure to sunlight, resulting in increased evaporation and mean maximum temperatures, both of which are conducive to drought stress in plants and invasion by aflatoxigenic fungi (Hill et al., 1983; Jones et al., 1981; Klich 1987). High population densities of *A. flavus* (both L and S strains) occur in nonagricultural soils of the hot, arid Sonoran Desert of the southwestern United States where the seeds and pericarps of native leguminous trees are extensively invaded and contaminated with aflatoxins (Boyd and Cotty, 2001). Therefore, desert environments may represent the preferred native habitat of *A. flavus*, and agricultural practices in nonarid regions may to some degree create localized desert-like conditions favorable for aflatoxigenic fungi.

Numerous studies have failed to demonstrate any short-term effect of crop rotation on soil populations of aflatoxigenic fungi (Angle et al., 1982; Cardwell and Cotty, 2002; Griffin and Garren, 1974; Griffin et al., 1981; McGee et al., 1996; Orum et al., 1997; Shearer et al., 1992). Soil populations may instead depend upon the long-term effects of periodic droughts on susceptible crops. Horn and Dorner (1998) speculated that differences in soil density of *A. flavus* among four major peanut-growing regions in the United States could be explained by climatic differences in the frequency of drought. During drought years that are associated with extensive aflatoxin contamination, combine harvesting of corn releases prodigious numbers of conidia into the air and scatters *A. flavus*-infected debris onto the soil

surface (Hill et al., 1984; Jones et al., 1981; Wicklow et al., 1984). Soil population densities of *A. flavus* may increase many-fold immediately following harvest (Wicklow et al., 1984). Horn et al. (1995) examined the effects of corn and peanut cultivation on soil populations of *A. flavus* and *A. parasiticus* in three fields in the southeastern United States. Population densities of both species remained fairly stable, with the exception of a corn field that was exposed to drought (Figure 5). In that instance, soil densities of *A. flavus* increased from 200 to 6400 CFU/g following harvest. Infected drought-stressed plants, therefore, periodically replenish soil populations of aflatoxigenic fungi. This population fluctuation was observed in 40 Iowa corn fields where soil populations during the drought year 1988 averaged 1200 CFU/g following harvest and subsequently dropped to 700 and 396 CFU/g by 1989 and 1990, respectively (Shearer et al., 1992). Those same fields were not exposed to further drought and consequently, densities continued to decline to 14 CFU/g in 1991 and 0.3 CFU/g by 1993 (McGee

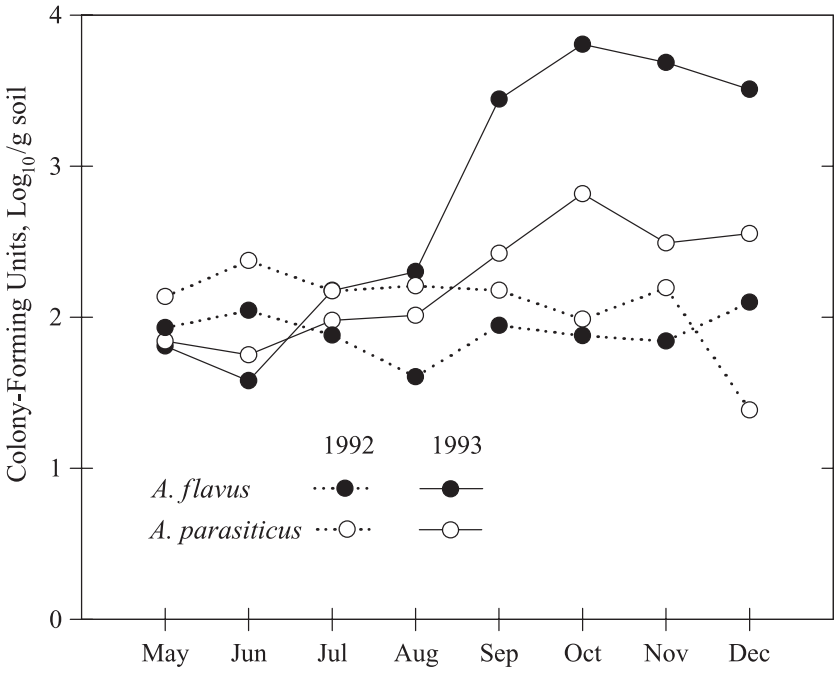

Figure 5. Soil populations of *A. flavus* and *A. parasiticus* from two cornfields in southwestern Georgia, USA. Corn was combine-harvested in August following nondrought (1992) and drought (1993) conditions. Horn et al. (1995).

et al., 1996). Infrequent droughts also may account for the changes in the genetic composition of *A. flavus* populations in soil as reported by Bayman and Cotty (1991). The decrease in soil populations between droughts may result in a low population base whose genetic composition has changed over time. Therefore, drought-induced *A. flavus* outbreaks in a single field are, to some degree, independent of one another due to their origins from different soil populations that serve as a source of primary inoculum.

In addition to the direct dispersal of conidia to soil during harvest, debris from crops such as corn, peanuts and tree nuts can support colonization and sporulation by aflatoxigenic fungi once deposited onto soil (Doster and Michailides, 1994; Griffin and Garren, 1976; Lussenhop and Wicklow, 1990; McGee et al., 1996; Shearer et al., 1992; Wicklow et al., 1984; Zummo and Scott, 1990). Angle et al. (1982) reported that 40 and 85% of soil populations of *A. flavus* and *A. parasiticus*, respectively, were associated with corn residues in Missouri, USA. However, it was not known whether these residues arose from crops infected before harvest or from noninfected debris that was subsequently invaded by soil inhabitants. Aspergilli are important decomposers of organic matter in soil (Klich et al., 1992) and several studies suggest that invasion of crop debris directly from soil contributes to soil populations. Griffin and Garren (1976) showed that rye, which is often planted as a winter crop before peanuts, led to an increase in *A. flavus* populations when deep-plowed into soil during the following spring. Similarly, root segments of peanut, cotton, soybean, snapbean and sorghum became colonized by *A. flavus* when buried in soil (Stack and Pettit, 1984b).

Because *A. parasiticus* primarily invades subterranean peanuts and rarely infects aerial crops, the relationship between crop infection and soil populations differs from that of *A. flavus* in several important respects. *A. parasiticus* appears to be more adapted to the soil environment than *A. flavus* (Diener et al., 1987; Horn et al., 1995) and as a consequence, exhibits less dependence on crop infection for maintaining soil populations. Soil populations of *A. parasiticus* can be relatively high in areas where peanuts are not cultivated and where the low infection rate of aerial crops cannot account for the population levels (Angle et al., 1982; Horn and Dorner, 1998; Wicklow et al., 1998). Even in peanut fields where infection with *A. parasiticus* contributes to soil populations, other environmental parameters often appear to predominate. Carter et al. (1998) reported that in the peanut-growing region of southeastern Australia, the proportion of *A. parasiticus* to *A. flavus* in soil has increased over a 20-year period from 1:1 to 10:1. The high genetic diversity of *A. parasiticus* strains from this entire period suggests that the increase was due to a build-up of native strains rather than the preponderance of an aggressive, newly introduced

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strain. Since peanuts had been grown in the region for more that 70 years, it was further hypothesized that the population increase was the result of changing environmental conditions or cultivation practices rather than due solely to the presence of peanuts. The peanut-growing region studied had suffered from protracted drought conditions during the 1990s that may have favored *A. parasiticus*, and the introduction of winter crops may have lowered soil levels of *A. flavus* relative to *A. parasiticus* whose populations are better adapted to survival in soil. Horn et al. (1994) reported that the proportion of *A. parasiticus* to *A. flavus* in soil inexplicably increased from 3:7 at planting to 1:1 upon instigation of drought despite a low incidence of seed infection by *A. parasiticus*. Populations of aflatoxigenic fungi in the geocarposphere have been reported to remain unchanged (Kloepper and Bowen, 1991; Griffin, 1972) or increase (McDonald, 1969; Subrahmanyam and Rao, 1977) during pod development. Those studies showing population increases have not distinguished between the direct effect of pods on the geocarposphere and the effect of established pod infections that secondarily produce conidia.

SELECTIVE EFFECTS OF CROPS

Aflatoxigenic fungi in agricultural ecosystems have two components to their life cycle, one as colonizers of organic matter in soil and the other as facultative parasites of crops. A comparison of the population structure of *A. flavus* and *A. parasiticus* with other fungal species may provide insight into the selective forces influencing the different life-cycle stages. Soil populations of *A. flavus* and *A. parasiticus* and soil populations of non-pathogenic *Fusarium oxysporum* demonstrate extremely high VCG diversities (Gordon and Okamoto, 1991; Horn and Greene, 1995). In contrast, *F. oxysporum* isolates pathogenic to crops have a low VCG diversity due to strong selection for pathogenicity in strains (Bosland and Williams, 1987; Larkin et al., 1990). This suggests that *A. flavus* and *A. parasiticus* have population characteristics consistent with a saprobic existence, with diversity perhaps being maintained in the soil environment, and that genetic diversity is not diminished through infection of crops (Horn et al., 1996). Bayman and Cotty (1991) and Horn and Greene (1995) reported considerable overlap between the *A. flavus* and *A. parasiticus* VCGs present in soil and those infecting crops (Figure 1).

Although genetic diversity within a species is often similar between soil and crop populations, diversity among species between the two populations can be quite different. The most striking example is the preferential infection of corn by *A. flavus* (Hill et al., 1985; Lillehoj et al., 1980). Horn et al.

(1995) reported that despite an equal proportion of *A. flavus* and *A. parasiticus* in soil, corn ears were infected only with *A. flavus*, which resulted in a large increase in soil populations of *A flavus* relative to *A. parasiticus* following harvest (Figure 5). Since *A. flavus* and *A. parasiticus* are both carried to corn by soil insects (Lillehoj et al., 1980), selection for *A. flavus* likely occurs in the crop. This is supported by research showing that *A. parasiticus* readily infects corn when artificially inoculated alone (Wilson et al., 1986; Zummo and Scott, 1990) but is out-competed by *A. flavus* when the two species are coinoculated (Calvert et al., 1978). Even in peanuts, a crop that is commonly infected with *A. parasiticus*, *A. flavus* appears to be the more aggressive species (Hill et al., 1985; Horn et al., 1994).

A continuing question concerning the population structure of aflatoxigenic fungi in agricultural ecosystems is: do certain crops select for aflatoxigenicity? Schroeder and Boller (1973) examined *A. flavus* populations from different crops in Texas and reported that 96% of isolates from peanuts produced aflatoxins as opposed to 79, 49 and 35% in cottonseed, sorghum and rice, respectively. The aflatoxin-producing ability of soil isolates from those same fields was not examined, which would indicate whether certain strains were preferentially selected from the soil population. Horn and Dorner (1999) postulated that the high density *A. flavus* in soil from the peanut-growing region of southern Alabama and Georgia is due to seed infection resulting from frequent droughts and that the high percentage of aflatoxigenic isolates (Figure 4) is the result of selective forces imposed by peanuts. Nevertheless, most crops are colonized by a mixture of aflatoxigenic and nonaflatoxigenic strains of *A. flavus* (Schroeder and Boller, 1973; Wicklow et al., 1998), and nontoxigenic strains applied to soil for biological control readily compete with native aflatoxigenic strains for available infection sites (Cotty, 1994; Dorner et al., 1998, 1999). Further research is necessary to resolve any possible crop effects on the aflatoxin-producing potential of *A. flavus* populations.

CONCLUSIONS

Any explanation for the role of aflatoxins in the life cycle of *A. flavus* must address the apparent lack of a selective advantage in crops for strains that produce aflatoxins. Aflatoxin production instead may be of importance in soil habitats where ecological niches are more diverse than in crops and where strains may be partitioned in some manner according to their aflatoxin-producing ability. Adverse environmental conditions likely associated with the soil environment, including elevated temperature, low pH and nutrient deprivation, help maintain aflatoxin-producing

ability in *A. flavus* over successive generations (Horn and Dorner, 2002). Insight into not only the role of aflatoxins in the fungal life cycle but ultimately, effective ways to control aflatoxin contamination of crops, may rest with soil.

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